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(54) Title: REDUCTION OF ADHESIONS USING CONTROLLED DELIVERY OF ACTIVE OXYGEN INHIBITORS

(57) Abstract

SOD and other active oxygen inhibitors are directly applied in combination with a barrier material at local sites of tissue injury to prevent or decrease formation of adhesions and undesirable profileration of cells. Preferred barrier materials are polymeric hydrogels providing controlled release of AOI which are directly applied to the afflicted tissue. Examples demonstrate the effects of SOD on pelvic adhesions in the rat when administered by intraperitoneal (I.P.) bolus and by localized sustained release from a topically applied hydrogel system.

REDUCTION OF ADHESIONS USING CONTROLLED
DELIVERY OF ACTIVE OXYGEN INHIBITORS

Background of the Invention

The present invention is generally in the area
5   of prevention or reduction of surgical adhesion.
Adhesions are a common complication of
surgery. They may develop in a variety of areas in
the body, and are characterized in that tissues
which were separate before surgery become bonded
10   together in the process of healing. The type and
degree of damage caused by adhesions is variable,
ranging from life-threatening, as in the intestines
due to blockage, to extremely disabling, as in
tendons or spinal cord, to chronic pain and
15   infertility in the pelvic cavity, to being
obstructive of further surgery in the pericardium.
Postoperative formation of pelvic adhesions remains
a serious problem in patients undergoing
gynecological surgery and is a principal cause of
20   infertility. In general, the most common causes of
pelvic adhesions in women are prior surgery,
endometriosis and pelvic inflammatory disease.
Injury to intact peritoneum as a result of
surgical insult or infection begins a cascade of
25   pathophysiological events. Within three hours of
surgical insult or infection, there is damage to
vasculature, resulting in increased vessel
permeability, and an inflammatory response,
resulting in further vascular damage due to
30   ischemia and reperfusion injury. The exudate of
serosanguionous fluid and fibrin matrix leads to
further ischemia, resulting in persistence of
fibrin matrix and collagenous adhesions, as well as
fibrinolysis to yield fibrin split products,
35   absorption of fibrin matrix, and normal repairs.
This cascade includes the expected events
associated with inflammation including both

neutrophil and macrophage migration to the site of inflammation. Associated with this cell migration is a rapid respiratory burst leading to the generation of oxygen radicals at the site of

5   inflammation. In the absence of sufficient free radical scavengers, high concentrations of oxygen radicals are capable of damaging the surrounding intact cells, including those responsible for vascular integrity. This increased permeability of

10   blood vessels can lead to exudation of proteinaceous serosanguinous fluid which serves as a matrix for fibrinous adhesions. In addition, increased vascular permeability leads to local interruption of blood flow and eventual cell death

15   in the vessels comprising the vascular supply. Reperfusion of tissues, following this ischemic event, leads to further generation of oxygen radicals and, ultimately, further exacerbates the degree of fibrinous adhesion formation.

20   Under normal circumstances, the fibrinolytic capacity of plasminogen activator activity (PAA) leads to the absorption of such fibrinous deposits and to conventional peritoneal healing. However, in the presence of severe tissue injury (e.g.

25   following surgical trauma), a decrease in PAA leads to abnormally persistent fibrin deposits and, ultimately, mature collagenous adhesions. Meticulous dissection (i.e. adhesiolysis) continues to be the most widely accepted treatment for

30   existing adhesions. A substantial fraction of surgery therefore requires follow-up surgery to repair the effects of the adhesions. This procedure is generally called "adhesiolysis"; in some organ systems, the procedure has specific

35   names, such as "tenolysis" in the freeing of tendons.

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The list of potential therapeutic modalities
used in prevention of formation and reformation of
adhesions is extensive and includes infusion of
liquids into the pelvic cavity at the time of
5   surgery, mechanical barriers between two opposing
surfaces, and intravenously injected or topically
applied pharmacologic agents (Tulandi, "Effects of
Ringer's Lactate on Postsurgical Adhesion. In:
Diamon, et al., eds, vol. 381, Progress in Clinical
10   and Biological Research (NY, Wiley-Liss 1993) 59-
63; Schwartz, et al., Sem. in Repro. Endocrin.
9:89-99 (1991); Pjilman, et al., Eur. J. Ost & Gyn.
Repro. Biol. 53:155-163 (1994); and Monk, et al.,
Am. J. Obstet. Gynecol. 170:1396-1403 (1994).
15   However, the incidence of symptomatic adhesion
formation remains high, and the clinical need for
adhesion prevention still exists.

Therapies of various sorts have been used to
prevent the initial formation of adhesions
20   ("primary" adhesions). These include lavage with
water-soluble polymers and/or biologically active
molecules ("drugs"), which are usually not very
effective. However, the use of superoxide
dismutate (SOD) combined with catalase prevented or
25   diminished endometriosis-induced adhesions in
rabbits in a study by Poretz et al, (Int. J.
Fertil. 36: 39-42, 1991).   Permanent mechanical
barriers, such as Teflon™ sheets, can be effective
but are difficult to remove; and degradable
30   barriers such as oxidized cellulose (InterCeed™,
Johnson & Johnson) and degradable polymeric gels
(Sawhney et al, 1993; Hill-West et al 1994) can
have significant utility in the prevention of
primary adhesions. Tsimoyiannis et al (Acta Chir.
35   Scand. 155: 171-174, 1989) reported reductions of
about 50% in the incidence and 50-70% in the
severity of ischemia-related induction of primary

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adhesions in rats, after administration of SOD,
catalase, DMSO (dimethylsulfoxide) or allopurinol
as an intravenous bolus before surgery.

It has been hypothesized that the commonality
5   of these drugs is in their inhibition of the
pathway leading to oxidative damage to tissue.
SOD, catalase and DMSO each directly destroy active
oxygen species, such as superoxide, peroxide, or
hydroxyl radical; allopurinol is known to inhibit
10   the enzyme xanthine oxidase, which produces
hydrogen peroxide.   These compounds which directly
or indirectly inhibit the effect of active oxygen
species on tissue are referred to herein as "active
oxygen inhibitors", or AOIs.   Superoxide dismutase
15   (SOD, dimer MW = 31.5 kDa, tetramer = 67 kDa) has
been efficacious in the treatment of
ischemic/reperfusion events in a wide variety of
tissues including brain, kidney, and heart
(Schneider, et al., _Fr. Rad. Biol. & Med._ 3:21-26
20   (1987); Zimmerman, et al., _Am. J. Med. Sci._
307:284-292 (1994); Voogd, et al., _Fr. Rad. Biol. &
Med._ 11:71-75 (1991); Fridovich, _Arch. Bioch. &
Biophys._ 247:1-11 (1986); Kontos, et al. _CNS Trauma_
3:257-263 (1986)).   There is also evidence that
25   SOD can be effective in the prevention of pelvic
adhesions (Tsimoyiannis, et al., _Acta Chir. Scand._
155:171-174 (1989); Portz, et al., _Int. J. Fert._
36:39-42 (1991); O'Leary, et al., _Ann. Surg._
June:693-698 (1987)).   However, efficacy using SOD
30   has been limited, due to its rapid elimination from
the bloodstream (Petkau, et al., _Res. Commun. Chem._
Pathol. Pharmacol. 15:641-654 (1976); Odlund, et
al., _Pharmacol. Toxic_ 62:95-100 (1988)).   Improved
efficacy has resulted from strategies for
35   increasing SOD content in the bloodstream including
chemical modification to reduce the rate of
elimination (Pyatak, eta l., _Res. Comm. Chem. Path._

Pharm. 29:113-127 (1980); Hill-West, et al. _Obstet. Gynecol._ 83:59-64 (1994)) and frequently repeated injections (O'Leary, et al., 1987).

Removal of adhesions once formed is
5   substantially more difficult than prevention of adhesion formation. Unfortunately, formulations which effectively prevent primary adhesions (e.g., Hill-West et al, 1994) can be substantially less effective in preventing re-adhesion after
10 adhesiolysis ("secondary" adhesions). While the exact biological differences between primary and secondary adhesions are not known, it is possible that the formation of primary adhesions depends on the persistence of fibrin bridges between the
15 disjoint parts, which are subsequently colonized by other cells and develop into permanent vascularized tissue. Anything disrupting formation or stabilization of the initial fibrin bridge would tend to prevent primary adhesion formation.
20 Secondary adhesions, however, may be the result of the normal healing process applied to injured pre-existing tissue, i.e. the lysed primary adhesion. The healing process, while not yet understood in detail, involves the mobilization of several cell
25 types and the formation of new collagen, and typically has initial stages lasting for up to two weeks followed by several months of maturation to obtain full repair. Because of these differences, it is possible that treatments effective in primary
30 adhesion prevention will require enhancement to prevent reformation of adhesions after adhesiolysis.

In summary, there have been no reports of compositions totally effective in eliminating
35 adhesions, especially in patients in which the injury is repeated, as in the case of patients having had multiple surgeries.

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It is therefore an object of the present invention to provide a method and compositions for preventing adhesions following surgery or infection.

5   **Summary of the Invention**
SOD and other active oxygen inhibitors are directly applied in combination with a barrier material at local sites of tissue injury to prevent or decrease formation of adhesions and undesirable
10   proliferation of cells. Preferred barrier materials are polymeric hydrogels providing controlled release of AOI which are directly applied to the afflicted tissue.
Examples demonstrate the effects of SOD on
15   pelvic adhesions in the rat when administered by intraperitoneal (I.P.) bolus and by localized sustained release from a topically applied hydrogel system.

                   Brief Description of the Figures
20   Figure 1 is a bar graph of the percent efficacy in primary adhesions rat uterine horn model of treatment with hydrogel (‖‖‖), SOD alone (≡), and SOD locally delivered via the hydrogel (open bar), as compared with untreated animals
25   (solid bar), at dosages of 2000 U SOD/ml versus 10,000 U SOD/ml.
           Figure 2 is a bar graph of the percent efficacy in secondary adhesions rat uterine horn model of treatment with hydrogel (‖‖‖), SOD alone
30   (≡), and SOD locally delivered via the hydrogel (open bar), as compared with untreated animals (solid bar), at dosages of 2000 U SOD/ml, 5,000 U SOD/ml and 10,000 U SOD/ml.
           Figure 3 is a bar graph of the percent
35   efficiency of hydrogel (‖‖‖), SOD alone (≡), and

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SOD locally delivered via the hydrogel (open bar),
compared with untreated animals (solid bar), for
both primary (open bars) and secondary (solid bar)
adhesion models.

5   **Detailed Description of the Invention**
          As described herein, a method and compositions
providing for prevention or minimization of tissue
adhesions, especially secondary tissue adhesions,
is described where active oxygen inhibitors such as
10   SOD are locally applied to the injured area in a
hydrogel formulation. In the preferred embodiment,
the hydrogel is polymerized *in situ* by
photopolymerization of a biocompatible,
biodegradable macromer solution such as a
15   polyethylene glycol dilactide diacrylate.
Alternatively, or in addition, a barrier material
can be used in combination with the active oxygen
inhibitor, as well as controlled release means for
the active oxygen inhibitor ("AOI"). These
20   materials, including the hydrogels, are generally
referred to as "barrier materials" unless otherwise
specified.
          Barrier Materials:
          Primary Barrier Materials:
25   Barrier materials can be administered as
fluids, pastes, or other fluent forms, and locally
altered in the body to form conforming barriers at
or near the site of application, or as solid
barriers. The barrier materials should be
30   biologically benign, and in particular, should not
induce a severe local inflammatory response.
Essentially all implants cause a transient local
reaction after implantation. A benign, or
biocompatible, material does not provoke a
35   prolonged or escalating inflammatory response.
Preferred barrier materials should be degradable or

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should dissipate within a reasonable time by
exposure to natural body fluids. Preferred barrier
materials should also serve as a depot for
controlled release of AOIs over a period of hours

5   to weeks, to allow local delivery directly to
injured tissues.

A preferred barrier material is a gel, most
preferably a gel material which is absorbed *in
vivo*, whether by gradual dissipation into solution,

10   spontaneous hydrolysis, enzymatic degradation, or a
combination of these. Examples of suitable gels
are the Pluronic® poloxamer gel systems are
polyalkylene oxides, some members of which form
gels at body temperature but are liquid at room

15   temperature; and the photopolymerizable gels
described by Hubbell et al (WO 93/17669) and
Sawhney et al, J. Biomed. Mats. Res. 28, 831-838
(1994). Pluronic gel systems are known as
anti-adhesion barriers (U.S. Patent Nos. 4,911,926

20   and 5,135,751 to Henry et al; U.S. Patent No.
5,366,735 to Henry) and as drug delivery vehicles
(U.S. Patent No. 5,292,516 to Viegas et al), as are
the cited Hubbell materials.

Numerous other formulations are potentially

25   capable of serving as barriers with drug delivery
capability. These include the polymers of U.S.
Patent No. 4,938,763 to Dunn et al, U.S. Patent No.
5,108,755 to Daniels et al, U.S. Patent No.
4,913,903 to Sudmann et al, U.S. Patent Nos.

30   5,100,992 and 4,826,945 to Cohn et al, U.S. Patent
No. 5,219,564 to Zalipsky et al, U.S. Patent Nos.
4,741,872 and 5,160,745 to De Luca et al, U.S.
Patent Nos. 4,526,938 and 4,942,035 to Churchill et
al, U.S. Patent No. 4,888,413 to Domb, U.S. Patent

35   No. 4,511,478 to Nowinski et al, U.S. Patent No.
4,957,744 to della Vallee et al, U.S. Patent No.
4,925,677 to Feigen, U.S. Patent No. 4,994,277 to

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Hingham et al, U.S. Patent No. 5,364,622 to Franz
et al and U.S. Patent No. 4,804,691 to English et
al. Hyaluronic acid materials, including
hyaluronic acid gels and membranes, are also
5   suitable for local delivery of AOIs to injured
tissue.

Oxidized cellulose, administered as a fabric,
forms a gel in bodily fluids and can deliver drugs
(U.S. Patent No. 4,889,722 to Sheffield et al).
10   However, it is believed to somewhat inflammatory
and is therefore not preferred. Purely mechanical
barriers, such as "Teflon®" fluoropolymer sheets
and other non-degradable materials, are less
desirable, but may be suitable if combined with
15   other means for controlled local release of the
drug directly to the injured tissue.

Although less preferred, gel barriers which
are not readily degradable can be used. These
include materials such as agarose, crosslinked
20   polyacrylamide, gelled polyvinyl alcohol, gelled
or crosslinked HEMA (poly hydroxymethyl acrylate),
crosslinked dextran, and other known gelling
materials. These can be very biocompatible, and
are suitable for drug delivery.

25   The concentration of the gel-forming polymer
in a hydrogel is variable, as is well-known.
Suitable concentrations of polymer are those which
give a gel which has adequate mechanical properties
to persist at the site of application for at least
30   the desired period of treatment, which will
typically be days to weeks. Minimum effective
gelling concentrations of various polymers range
from as low as 0.1% (w/w) for ultrapure agarose, to
between 3 and 5% for the polymers of Hubbell et al,
35   to over 10% for some poloxamers such as Pluronics®.
Upper limits are imposed by solubility, by
viscosity, by the onset of brittleness of the gel,

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by excessive swelling after formation, by osmotic
effects of the applied monomers on tissue, and by a
desire to minimize the amount of polymer applied in
a treatment. These factors and their manipulation
5   are known in the art, and vary with the polymer.
Upper concentration limits may vary from as low as
2% for agarose, to between 25 and 30% for the
polymers of Hubbell, to 50% or more for poloxamers.
        Preferred materials for barriers and delivery
10 means are characterized by certain critical
characteristics, including: biocompatibility;
delivery times extending from a day up to weeks;
compatibility with the AOI to be delivered; and, in
more preferred embodiments, biodegradability.
15 Preferred materials contain at least some water,
further containing physiologically-acceptable salts
and buffers; higher levels of water are preferred,
where otherwise compatible with mechanical and
diffusional properties of the materials. It is
20 preferred to combine the barrier function with the
controlled-release function in a single material;
of such materials, hydrogels are preferred.
        As shown in the Examples below, a preferred
mode is the incorporation of an AOI, preferably
25 SOD, in a solution also containing a biocompatible,
biodegradable, gel-forming photopolymerizable
monomer, and suitable photoinitiators, buffers and
stabilizers; delivery of the combined solution to a
site where adhesiolysis has been performed, and
30 covering the affected areas; and photopolymerizing
the composition to create a barrier gel which
slowly releases the AOI during a period of days to
over a week.
        As noted above, the concentration of the
35 polymerizable material is variable. For the
particular material used in the examples, the
preferred concentration of the monomer is in the

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range of 10% to about 25% w/w in buffered isotonic
saline solution. Higher concentrations tend to
give slower and more prolonged release of the AOI,
and to last longer as barriers, but are
5 correspondingly more viscous, which can make
application more difficult if done through a
laparoscope or catheter. Other materials will have
different preferred concentrations, as previously
noted.

10   Drug delivery means.

         Controlled delivery means can be employed in
addition to a gel, or in place of a gel. There are
a large number of physiologically-acceptable drug
delivery materials known in the art which have the
15 following properties:

         1.   The delivery means must be localizable,
or at least potentially localizable, to allow the
delivery of the contents primarily to a particular
target organ or region. A suitable means, although
20 not preferable for human use, is an osmotic pump,
such as an Alzet® pump (Alza Co.)

         2. The delivery means must be biocompatible,
and in particular should not be substantially
inflammatory in the body. Illustrative controlled
25 delivery means include small particles of drug
entrapped in a bioerodible polymer such as
polyglycolide, polylactide or polyanhydride, for
example, U.S. Patent No. 4,898,734 to Mathiowitz et
al; U.S. Patent No. 5,175,235 to Domb; U.S. Patent
30 No. 5,286,763 to Gerhart et al; and U.S. Patent No.
4,745,161 to Saudek et al, optionally coated with a
release-retarding shell, which are in turn
delivered to the site at which adhesions are to be
prevented, preferably with additional means to
35 retain them near the site, such as a barrier
membrane or a gel. Controlled delivery means are
especially important when the drug to be delivered

has a high aqueous solubility and a low molecular weight.

    3. Preferably, the delivery means will degrade in the body, concurrently with delivery of drugs or
5   subsequently. Most of the polymers noted as potential hydrogel materials above are biodegradable, as are the polymers listed in the previous paragraph. Classical erodible polymers, such as poly(hydroxyacids) (e.g., polyglycolide,
10 polylactide, and polycaprolactone) are suitable in many situations, although they are somewhat more inflammatory than some newer polymers.

    Pharmaceutically Active Compounds
    Active Oxygen Inhibitors.
15   As used herein, AOIs are defined as compounds which destroy, or prevent the formation of, active oxygen species. Active oxygen species include superoxides, peroxides in general, hydrogen peroxide, and hydroxyl radical (OH). Other active
20 species derived from active oxygens, such as hypochlorite ion (OCl-), hydroxyl free radicals on carbohydrates, "singlet oxygen", or ozone, are also included. The active oxygen species damage tissue directly. Moreover, they may cause indirect damage
25 by attracting cells to the site of injury, or otherwise stimulating an inflammatory response.

    A preferred antioxidant drug is superoxide dismutase (SOD). It is thought that SOD prevents tissue damage and inflammation by destroying the
30 superoxide radical. Any of the forms of SOD are suitable. Sources include human, which is preferred for minimization of immunogenicity, and also other known sources including bovine and bacterial; SOD from various tissues, such as liver,
35 red cells, and others; SOD having any functional metal ion, such as manganese, iron, copper, and zinc. One form (manganese) of recombinant human

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SOD is described in U.S. Patent No. 5,260,204 to
Heckl et al, referencing EP-A 138111 to Chiron as
a source of recombinant human copper/zinc SOD. Also
included are SOD modified with pendant polymers,
5   such as polyethylene glycol or polyvinyl alcohol;
natural SOD produced by recombinant or transgenic
processes; and variant forms of SOD produced by
mutation or protein engineering. All of these
processes are known in the art.
10   Alternative methods of delivery of SOD, or of
other protein-based drugs of the antioxidant class,
to the site include expression by transformed cells
instilled at the site, and transient transformation
of cells at the site by recombinant DNA including a
15   mammalian promoter, such as a plasmid, an
expression cassette, or a viral vector. These may
be delivered locally by any suitable means,
including liposomes, polymeric composites, in
solution via catheters and other similar devices,
20   via electroporation or iontophoresis, and by other
methods known in the art.   Other drugs having
antioxidant activity (i.e., destroying or
preventing formation of active oxygen) may be used
in the prevention of adhesions, either in
25   combination with SOD, in combination with each
other, or alone. Protein drugs include catalases,
peroxidases and general oxidases or oxididative
enzymes such as cytochrome P450, glutathione
peroxidase, and other native or denatured
30   hemoproteins. Most such molecules have detectable
peroxidase activity. Small-molecule drugs may act
by directly absorbing or inactivating active oxygen
species. These include vitamin C (ascorbic acid)
and vitamin E (tocopherol), food-approved
35   antioxidants such as BHT and BHA (butylated
hydroxytoluene, butylated hydroxyanisole), phenolic
compounds and quinones, and highly hindered

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nitrogen free-radical scavengers such as
2,2,6,6-tetramethyl piperidine. Other drugs act by
altering enzyme activity, and these may be very
effective. Allopurinol, which inhibits the enzyme
5   xanthine oxidase, substantially prevents the
generation of superoxide, and has been found to be
highly effective. Other xanthine oxidase
inhibitors are also expected to be efficacious.
Verapamil is known as a calcium-channel blocker,
10   and is used as a vasodilator. It is highly
effective in prevention of primary adhesions in the
models described in the examples. Its mechanism of
action is unknown, but it may prevent the
stimulation of cells such as macrophages, which are
15   believed to secrete active oxygen when stimulated.
As defined herein, verapamil is an inhibitor of
active oxygen.

     Not all materials effective in prevention of
adhesion formulation act against active oxygen.
20   Other materials such as fibrinolytics can be used,
including urokinase, tissue plasminogen activator,
and ancrod. These materials alone may be
effective, for example, as described by Dunn, et
al., Am. J. Obstet. Gynecol. 164:1327-1330 (1991)),
25   but enhanced when in combination with AOIs.

     Formulations

     The amount of AOI administered to the site
will be adjusted to the particular site, and to the
nature of the controlled release means being
30   employed. A suitable starting amount is a single
dose; this may then be adjusted upwards or
downwards to optimize the amount. The results
shown in the Examples below indicate that a broad
optimum or plateau exists for SOD; many suitable
35   AOIs, in other studies, also show broad
effectiveness regions. The prolonged presence of
low levels of the inhibitor at the injured site,

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provides not only protection in the immediate
aftermath of surgery, but also minimizes damage and
inflammation during at least the early stages of
the healing process, and generally permits the use
5   of lower effective doses.

The formulation of AOI and barrier materials
should deliver AOI directly to the injured tissue
over a period of days from about 0.5 day up to
about 20 days under *in vivo* conditions. This is
10   preferably controlled by the gel incorporating the
AOI, but may also be provided by a mechanical
barrier such as a membrane or fabric. The
formulation may also contain additional means for
regulating the release of the inhibitor, such as
15   microspheres, microcapsules, microparticles or
liposomes (referred to jointly herein as
"microspheres"), especially when the AOI is a small
molecule of less than about 20,000 daltons, where
the AOI is released from the microspheres into the
20   gel, from which it diffuses into the tissue, alone
or in combination with release of AOI from the gel.
Other means for controlled release include intact
or comminuted gels, polymers with AOIs releasably
bound thereto, and slowly dissolving particles of
25   the AOIs or a complex thereof with an inert salt or
organic molecule. For example, verapamil as the
free base is practically insoluble in water, while
its hydrochloride is very soluble. Enclose of the
liquid free base form in conventional timed-release
30   microcapsules can be used to provide extended
periods of delivery, by incorporating the
microcapsules into the polymeric barrier.

The formulations may further contain
excipients, buffers, stabilizers, and other common
35   additives, as known in the art of pharmaceutical
formulation. The formulations may also contain
ingredients needed for the formation of the

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barrier, such as polymerization-inducing or
polymerization-enabling materials. These may
include photoinitiators, chain transfer reagents,
and co-monomers.

5   The formulation may be prepared in more than
one container, for mixing just before use, and may
be stabilized by lyophilization, freezing,
desiccation, refrigeration, or other commonly used
means. The formulation must be sterile; where
10   ingredients of the formulation are unstable, filter
sterilization and aseptic filling are preferred.

Methods of Treatment

Application of Formulations to Tissues

The formulation can be applied to the affected
15   tissues by any suitable means. These include
spraying, washing with a medication-containing
fluid, spreading with an instrument or by hand, and
direct implantation of a preformed inhibitor-
containing barrier. When the barrier is a gel or
20   other solid form, the gel may be implanted;
preferably, however, a solution of the mixture of
gel precursor and inhibitor to be delivered is
delivered to the site of application, and gelled
directly onto the tissue by an appropriate means.
25   Preferred gelling means, because of their
simplicity, are physical gelation due to a change
in temperature or to a cessation of shear; and
photopolymerization of a photoreactive gelling
monomer. Chemical redox reactions, ionic
30   crosslinking, and other methods of gel formation
can also be utilized.

Instruments for performing delivery and
gelling functions will be selected for the
particular application, using principles and
35   instruments known in the art. These will typically
include catheters or laparoscopes for minimally
invasive access to interior sites of the body;

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syringes for surface or surgically exposed sites
such as in tendon or wound repair; and appropriate
light sources when a polymerization reaction is
required. Some suitable instruments are described
5   in detail in U.S. Patent No. 5,328,471, U.S. Patent
No. 5,213,580, and U.S. Serial Nos. 08/054,385 (WO
94/24962), 08/036,128 (WO 94/21324), and
08/265,448, which are hereby incorporated by
reference.

10   Medical indications

Treatment with the compositions, medications
and methods described herein is intended for any
site in which adhesions form and have potential or
actual deleterious effects. These include primary,
15   and especially secondary, adhesions in the
following: in the abdominal cavity, including
intestine to intestine, and intestine to
peritoneum; in the pelvic cavity, including
adhesion of the uterus, ovaries or fallopian tubes
20   to other structures including each other and the
pelvic wall; in tendons and their support
structures, including tendon to pulley or to
synovium; in the repair of nerve sheaths; in repair
of the spinal column or disks; in the pericardium;
25   in treatment of joints for inflammation and to
prevent pannus formation; and in any situation in
which adhesions form which impair function or cause
pain. Moreover, the compositions may be used in
other conditions in which an unwanted tissue
30   proliferation occurs. These can include restenosis
of arteries, repair of keloid or hypertrophic
scars, hypertrophy which obstructs ducts, such as
benign prostatic hypertrophy, and endometriosis.

The present invention will be further
35   understood by reference to the following non-
limiting examples. The following studies were
designed to (1) demonstrate the potential for using

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locally delivered superoxide dismutase and (2) to
compare the efficacy of hydrogel-delivered SOD with
hydrogel alone or SOD alone in the prevention of
postsurgical adhesions.

5   **Example 1:   Release of model compounds.**

The compatibility of SOD protein with the
components of the FocalGel system was tested *in
vitro*. The compatibility of SOD with each
component of the hydrogel system was tested

10   step-wise, testing for any effect on SOD by gel
electrophoresis, reversed phase HPLC, capillary
electrophoresis and an enzymatic activity assay
(cytochrome C). Since photopolymerization of the
gel requires UV light, the effect of UV exposure

15   (340-400 nm) on SOD stability was also evaluated.

Hydrogel Macromer (pre-polymer) preparation

In all pre-development studies, hydrogel was
prepared in the same manner using the following
method: Pluronic® F127 was added to a solution of

20   potassium monobasic phosphate at pH 6.8.
Photoinitiator (Irgacure® 651) was dissolved in
tertiary butanol and added to the Pluronic®/buffer
solution. Macromonomer and polyethylene glycol
(PEG) 8000 were then added. Following sterile

25   filtration, vials were filled and the water and
t-butanol were removed by lyophilization. Prior to
use in these studies, each vial of hydrogel was
reconstituted with normal saline to arrive at a
macromonomer/PEG 8000 concentration of 10% w/w, 3%

30   w/w Pluronic®, and 1200 ppm Irgacure® 651.

A.   Formulation Compatibility Studies.

SOD compatibility in the hydrogel formulation
was evaluated using a step-wise study design. SOD
stability in each formulation component was tested

35   in the presence and absence of ultraviolet light
exposure (365 nm). In these studies, protein
stability was evaluated by gel electrophoresis

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(SDS-PAGE and native PAGE), HPLC, capillary
electrophoresis and enzymatic activity by
cytochrome C reduction.

Control samples were prepared by diluting one
vial of SOD (15,000 U, 5 mg) with 1.5 mL PBS to
give a final [SOD] = 10 KU/mL. Additionally, 400
μL of this solution was diluted to 1 mL with PBS to
give a final [SOD] = 4 KU/mL.
Irgacure®/F127-treated samples were prepared by
adding 1.5 mL Irgacure®/F127 (1200 ppm and 3% w/w,
respectively) to 1 vial of SOD (final [SOD] = 10
KU/mL). For all UV-treated samples, 300 μL of
sample solution was illuminated for either 20 or 60
seconds. Hydrogel-treated samples were prepared by
adding 1.0 mL of hydrogel solution to a vial of SOD
to give a final [SOD] = 15 KU/mL. 200 μL of this
solution was photopolymerized for either 20 or 60
seconds after 1.5 mL of PBS was added to the
hydrogel. Samples were placed in an incubator at
37°C for 24 hours prior to analysis.

Samples for electrophoresis were prepared by
diluting 100 μL of sample with 300 μL of sample
buffer (Bio-Rad technical manual LIT-188 REV C).
Samples were not heated prior to analysis. 10 μL
of sample was loaded onto the gel except for
samples where SOD was released from hydrogel (20 μL
used in these cases). Standards were run according
to the vendor's protocol. Molecular weight bands
in electrophoretic gels were integrated by image
analysis.

There appears to be two low molecular weight
subunit forms that are not identical, with the bulk
of the protein existing as a tetrameric structure.
To give a baseline for relative compatibility
comparisons, SDS-PAGE of SOD was initially
performed using a saline (no exposure) control.
The data suggest that 92% of the protein exists as

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the high molecular weight form (67 kDa) with the
remaining 8% existing as the low molecular weight
monomer species (15.5 kDa). There was also a 12.7
kDa peak present, but the band intensity was very
5   low and therefore not quantitated. Upon addition
of the initiator and macromer formulation
components, the relative band intensities remained
nearly constant, and, with the addition of UV
exposure, no visible changes were observed.
10   Native-PAGE analysis of SOD control solutions
(SOD in PBS pH 7.4, [SOD] = 1.33 and 3.33 mg/mL)
shows single band SOD migration with band intensity
directly related to protein content.
Irgacure®/F127-treatment and hydrogel-extraction
15   solutions display similar SOD band migrations and
intensities as those displayed by the control.
         SOD was incorporated into the initiator
solution (3% F127 and 1200 ppm Irgacure®) by
pipetting 2 mL of initiator solution into one vial
20   containing SOD (final [SOD] = 7.5 KU/mL). 300 μl
aliquots of SOD/initiator were illuminated under
the Black Ray lamp (power output = 10 mW/cm2,
lmax=365 nm). The results of analysis were
analyzed as the total area of the SOD peaks,
25   calculated SOD amount, expected SOD amount and
percent recovery. Irgacure® levels in control
solutions were also determined and compared to
levels observed in the SOD/initiator samples.
         SOD was also incorporated into hydrogel via
30   bulk gel polymerization using 2 and 5 minute
exposure times. Following polymerization, SOD was
extracted from 200 μL bulk gels (8 mm x 10 mm) over
two days in PBS. Each of the extraction samples
was analyzed for SOD amount and the cumulative
35   amount was calculated as the % recovered of
initial. The peak area of SOD in Irgacure®/F127
remained constant compared to the non-illuminated

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control after UV exposure (up to 5 min. exposure
time). The data also show that the degradation
products of Irgacure® photoinitiator in the control
solutions were not altered in the SOD/Irgacure®
5   treatment solutions. All degradation peaks
observed in the samples were accounted for by
Irgacure® degradation. Extraction of SOD from
hydrogel resulted in 86.4 and 78.2% of the expected
level recovered. The extraction was most likely
10   not complete because SOD has been shown to be only
partially released over 49 hours in vitro.
Chromatograms for SOD extracted from Hydrogel show
that peak distributions remained similar to those
in control solutions.
15   Capillary testing was performed. In this set
of experiments, a standard curve of SOD in water
was prepared (1.48 - 11.84 KU/mL). SOD
compatibility samples were prepared at 1.2, 1.6 and
11.9 KU/mL. Separation by CE shows that three
20   sharp peaks are present (3.15, 3.20 and 3.27
minutes). The third peak has a shoulder which is a
possible fourth peak. Each peak was integrated
separately and the third peak was integrated to
include the shoulder peak area. The standard curve
25   of total peak area versus SOD concentration shows
excellent linearity ($R^2 = 1.000$) over [SOD] = 1.48
to 11.84 KU/mL. The results show that the detected
levels of SOD in Irgacure®/F127 (no illumination)
were 130 and 82% of expected levels. When
30   SOD/initiator samples were illuminated, the SOD
levels remained high regardless of SOD
concentration. Results from low [SOD] samples (1.2
KU/mL) showed more response variability than those
prepared at higher concentrations (11.9 KU/mL).
35   SOD extracted from bulk photopolymerized Hydrogel
showed 89 - 108% of the expected SOD level
recovered and the peak distribution remained

similar to the SOD control.

Under standard assay conditions, cytochrome C (10^{-5} M) reduction was measured at 25°C with 5×10^{-5} M xanthine oxidase (XOD) in 50 mM potassium

5 phosphate buffer (pH 7.8) containing 0.1 mM EDTA. Inhibition of the reduction of cytochrome C by 50% was defined as 1 unit of SOD activity. Continuous spectrophotometric rate determination was performed over 5 minutes at 550 nm using a Hitachi UV/Vis

10 spectrophotometer. Compatibility solutions were prepared and treated at [SOD] = 5000 U/mL, then diluted with normal saline to a final [SOD] = 10 U/mL. Relative specific activity was determined by comparing treatment solution activity to control

15 solution activity ([control] = 10 U/mL having 50% inhibition of cytochrome C reduction activity).

SOD in normal saline (10 U/mL, no illumination) was used as a control sample for relative compatibility comparison. The theoretical

20 enzymatic activity as defined by the assay was 50% inhibition activity and the control solution showed the expected response (51.5% inhibition). Upon the addition of the initiator and/or macromer components, no dramatic change in the SOD activity

25 was observed. Additionally, when SOD/hydrogel formulations were exposed to 1 min. of UV exposure, SOD activity levels (50.9% inhibition) were retained.

In vitro Release Characteristics

30 Subsequently, bulk photopolymerized hydrogel devices containing four levels of SOD (1, 3, 5, and 10 KU/mL) were prepared and evaluated for SOD in vitro release kinetics. SOD was dissolved in each of the individual components of the hydrogel

35 prepolymer solution, in the presence or absence of ultraviolet light (10 mW/cm^2) and the SOD was then characterized by gel electrophoresis (SDS PAGE and

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native PAGE), reversed phase HPLC, capillary
electrophoresis and enzymatic activity assay (as
inhibition of cytochrome C reduction). No changes
in protein characterization or enzymatic activity
5   were observed using these techniques. The kinetics
of release of SOD from bulk hydrogels were
determined *in vitro* at hydrogel loads ranging from
1,000 to 10,000 U/mL.

10% macromer solutions were prepared using
10   1200 ppm Irgacure and 3% F127 in normal saline. To
parallel the *in vivo* study design, four doses of
SOD (1, 3, 5 and 10 KU/mL) were incorporated into
the macromer solution and bulk photopolymerized in
200 μL aliquots (365 nm, 10 mW/cm2). SOD release
15   in PBS was monitored over time using the micro-BCA
method; activity of released SOD was also
characterized using cytochrome C inhibition
analysis.

The hydrogel cross-link density and the
20   effective size of the molecular species are two
major determinants of the drug release kinetics out
of the hydrogel. In this case, three molecular
weight species of SOD (67 kDa oligomer, 15.5 and
12.7 kDa monomers) are present. Based on data
25   using dextran model compounds, it was expected that
the low molecular weight species would completely
elute from the gel within the first 12 hours and
medium molecular weight species (67 kDa) would
initially show a burst of release within the first
30   48 hours with prolonged release evident as the
superficial diffusion zone is depleted.

The release of SOD from hydrogel *in vitro* in
saline was biphasic. Hydrogels formed from 10%
macromer solutions released 90% of the incorporated
35   SOD within 48 hours ($t_{1/2}$ = 4 hours) with the
remaining 10% being released by zero-order kinetics
over the following 7 days (t_{100} = 8 days). In

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contrast to the data obtained with dextran, the
increase in the drug diffusion path length coupled
with the hindered diffusivity of the larger
molecular weight species results in extended
5   durations of release to 8 days. Specific activity
analysis of sample elution media demonstrates
extended formulation stability.

**Example 2:   Prevention of primary adhesions in
rat model.**

10   Animal Models

In vivo efficacy experiments were performed in
primary and secondary rat uterine horn adhesion
models (RUHAM) to compare the relative efficacies
of SOD when delivered by IP bolus versus controlled
15   hydrogel delivery. Control groups included
untreated (injured) and hydrogel (no SOD) animals.
The final *in vivo* efficacy studies used dose-range
study designs (1, 3, 5 and 10 KU/mL) in primary and
secondary RUHAM models. The endpoint used for *in*
20   *vivo* evaluation studies was 1 week post-treatment.

Experiments were performed using two rat
uterine horn models which develop adhesions in
response to an initial ischemic insult. The first
model generates *de novo* adhesions (primary model)
25   over a 7 day time period. The second model is a
reformation (secondary) model whereby the fibrinous
adhesions developed in the primary model are lysed
and reformation of adhesions is observed 7 days
after adhesiolysis. Forty sexually mature female
30   Sprague-Dawley rats (225-250 grams) were used in
the primary adhesion model. The animals were
anesthetized following intramuscular injection of 4
mL/Kg of a mixture of ketamine (25 mg/mL), xylazine
(1.3 mg/mL), and acepromazine (0.33 mg/mL).
35   Aseptic technique was used throughout the surgical
procedure. After preparing the abdomen, a 3
centimeter lower midline incision was made to
expose the pelvic cavity. The uterine horns were

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positioned to expose the vascular arcade. Ischemia
to the central portion of the uterine horns was
induced by cauterizing the vascular arcade using
bipolar electrocautery. Care was taken not to
5   cauterize the most anterior and posterior vessels
supplying blood to the horn to maintain minimal
blood flow and ensure organ viability. Two
additional injuries were made on the antimesenteric
surface of the horn approximately 2.5 cm apart
10   using electrocautery. Following surgical injury,
animals were assigned to one of the four treatment
groups in a random fashion. The musculoperitoneal
layers and the overlying fascia were closed with a
4-0 vicryl absorbable suture and the skin incision
15   closed using 7 mm stainless steel staples.

The animals were evaluated for adhesion
formation at the end of one week. The "percent of
adhesions" along the uterine horn is calculated as
the measured length of the uterine horns engaged in
20   adhesions divided by the entire length of the
uterine horn multiplied by 100. The "percent
efficacy" is 100 minus the "percent of adhesions."

In a second experiment, seven days after
initiation of the primary adhesion model, animals
25   (n=40) were opened and the uterine horns were
exposed once again. Using microscopic surgical
technique, adhesions between the uterine horns and
other organs were carefully dissected and lysed.
Bipolar electrocautery was used to maintain
30   hemostasis. Upon completion of adhesiolysis,
animals were assigned to different groups, treated
according to protocol, and the peritoneal cavity
was closed. Animals were evaluated at the end of a
one week time period for adhesion reformation, and
35   adhesion scores were calculated as described above.

In both the primary and secondary adhesion
models, animals were assigned randomly to one of

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four groups according to the following experimental
design:

Group 1:   Control injury with no further treatment

Group 2:   Hydrogel barrier alone

5   Group 3:   SOD alone

Group 4:   Hydrogel containing SOD

    For treatment Groups 2 and 4 (where Hydrogel
was required), formulations were prepared as
described above and 0.25 mLs of solution was

10   applied onto the medial and lateral surface of the
uterine horns for a total volume of 1 mL per
animal. The preparation was subsequently
photopolymerized by exposure to long wave
ultraviolet light delivering an nominal irradiance

15   of 20 mW/cm^2 for 20 seconds.

    In Group 3, lyophilized SOD reconstituted with
normal saline was dripped onto the surface of each
uterine horn in 0.25 mL aliquots as described
above. Four experiments were conducted to

20   determine the effect of dose on primary and
secondary adhesion formation. Doses ranging from
2,000 to 10,000 U/mL were compared when applied
directly to peritoneal tissue and when added as a
supplement to the Hydrogel formulation.

25   Results in Primary Adhesion Model.

    The results are shown in Figure 11. In all
"untreated" control animals (Group 1), 93% or more
of the uterine horn surface was adherent to
adjacent mesometrium and/or organs resulting in

30   efficacy scores of 7% or less.   Hydrogel applied
as a barrier to adhesion formation (Group 2) showed
a broad range of efficacy values ranging from 11 to
46% efficacy. Application of SOD solution (Group
3) resulted in efficacy scores of 86-93%. Efficacy

35   scores ranged from 79 to 85% when hydrogel
containing SOD was applied (Group 4). No
significant effect on efficacy was observed whether

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the bolus dose of SOD or SOD incorporated into
Hydrogel, respectively, was 2,000 (10000U/kg) or
10,000 U/mL (50,000 U/kg).

Example 3:   Prevention of adhesion reformation
5   **in rat model.**

Results in Secondary Adhesion Model.

The results are shown in Figure 2. In all
"untreated" control animals (Group 1), 94% or more
of the uterine horn surface was adherent to the
10   adjacent mesometrium or other organs resulting in
efficacy scores of 6% or less. Hydrogel applied as
a barrier to adhesion reformation (Group 2)
resulted in efficacy scores of 20 to 40%. When SOD
solution was applied (Group 3) scores ranged from 0
15   to 29%. Finally, application of SOD after
incorporation into Hydrogel (Group 4) resulted in
efficacy scores ranging from 67 to 94%. No
significant effect on efficacy was observed when a
bolus dose of SOD or a dose of SOD incorporated
20   into Hydrogel, respectively, was 2,000, 5,000, or
10,000 U/mL.

Since no significant difference was found as a
function of dose, the data from individual
experiments were combined to summarize differences
25   between treatment groups for both primary and
secondary models, as shown in Figure 3.

Diamond, et al., Fert. and Ster. 47:864
(1987); Peters, et al., Br. J. Obstet. Gyn. 99:59
(1992); Lucian, et al., Obstet. & Gyn. 74:220
30   (1989); Luciano, et al., Fert. and Ster. 48:1025
(1987); and Surrey, et al., J. Repro. Med. 27:658
(1982), have demonstrated that reduction of
secondary adhesions is more difficult than primary
adhesions in animal models. Based on the data
35   presented here, it is likely that primary and
secondary adhesions in rats are formed through
different pathologic sequences. When histologic
evaluations and the propensity for adhesion

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formation at the wound sites are compared, the
differences between the two models become apparent.
Lysis of fibrin followed by adhesion resorption
occurs from day 7 through 28 in the primary model.

5   In contrast, the secondary adhesion model results
in persistent collagenous adhesions that do not
resorb over an eight week period. These data
indicate that there is a need for a pharmacologic
intervention that lasts for a longer period of time

10   to prevent secondary adhesions than a single bolus
can provide. Adhesions formed in this secondary
model are qualitatively similar to those
experienced in the clinical situation. Although
other formulations of hydrogel have produced

15   efficacy in adhesion models, the particular
formulation and application method used for this
study provided marginal efficacy as a barrier.
However, SOD loaded into the same formulation or
the direct application of SOD solution to the

20   uterine horns showed much higher efficacy in the
primary model. This indicates that in this primary
adhesion model, efficacy was produced by SOD due to
early intervention in the pathologic sequence which
is consistent with the production of oxygen-derived

25   radicals during the initial stages of inflammation.
Moreover, since the rate of elimination of SOD from
blood is rapid, one may speculate that the efficacy
shown by the bolus dose occurred in a short time
frame. In the secondary model, there was a

30   dramatic decline in efficacy when the barrier alone
or a bolus of SOD was applied. However, a
significant level of efficacy was achieved when the
two modes of therapy were combined (i.e. the
barrier loaded with SOD). This supports the

35   hypothesis that more prolonged exposure to SOD is
required when treating lysed, mature collagenous
adhesions similar to those formed in the clinical

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population, rather than the short-lived fibrinous
adhesions formed in the primary model.

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We claim:

1.   A composition for the inhibition of cell proliferation at a tissue site, comprising an effective amount of one or more inhibitors of active oxygen species and a polymer capable of polymerizing to form a barrier material at the tissue site.

2.   The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

3.   The composition of claim 1, further comprising means for controlling the release of the inhibitor.

4.   The composition of claim 3 wherein the means for controlling the release is the barrier.

5.   The composition of claim 3 wherein the means for controlling the release is provided in addition to the barrier.

6.   The composition of claim 5 wherein the means for controlled release are selected from the group consisting of microparticles, microcapsules, liposomes, intact or comminuted gels, polymers with the inhibitors releasably bound thereto, and a complex of an inert salt or organic molecule with the inhibitor.

7.   The composition of claim 1, wherein the barrier material is pre-formed prior to application to a the tissue site.

8.   The composition of claim 1, wherein the barrier-forming polymer is a polymer capable of forming a hydrogel.

9.   The composition of claim 8, wherein the polymer is degradable in vivo.

10. The composition of claim 8, wherein the polymer is photopolymerizable.

11. The composition of claim 8, wherein the polymer is present in a range of 0.5% to 80% by weight of the composition.

12. The composition of claim 11, wherein the polymer is present in a range of 2% to 50% by weight of the composition.

13. The composition of claim 12, wherein the polymer is present in a range of 4% to 30% by weight of the composition.

14. The composition of claim 1, wherein the inhibitor is selected from the group consisting of superoxide dismutase, catalase, allopurinol, verapamil, and combinations thereof.

15. The composition of claim 14, wherein the inhibitor is superoxide dismutase.

16. The composition of claim 1 further comprising a biologically active compound other than active oxygen inhibitors.

17. A method for the inhibition of cell proliferation at a tissue site, the method comprising administering an effective amount of at least one inhibitor of active oxygen species in combination with a polymer capable of polymerizing to form a barrier material, as defined in claims 1-16, at the tissue site where cell proliferation is to be inhibited.
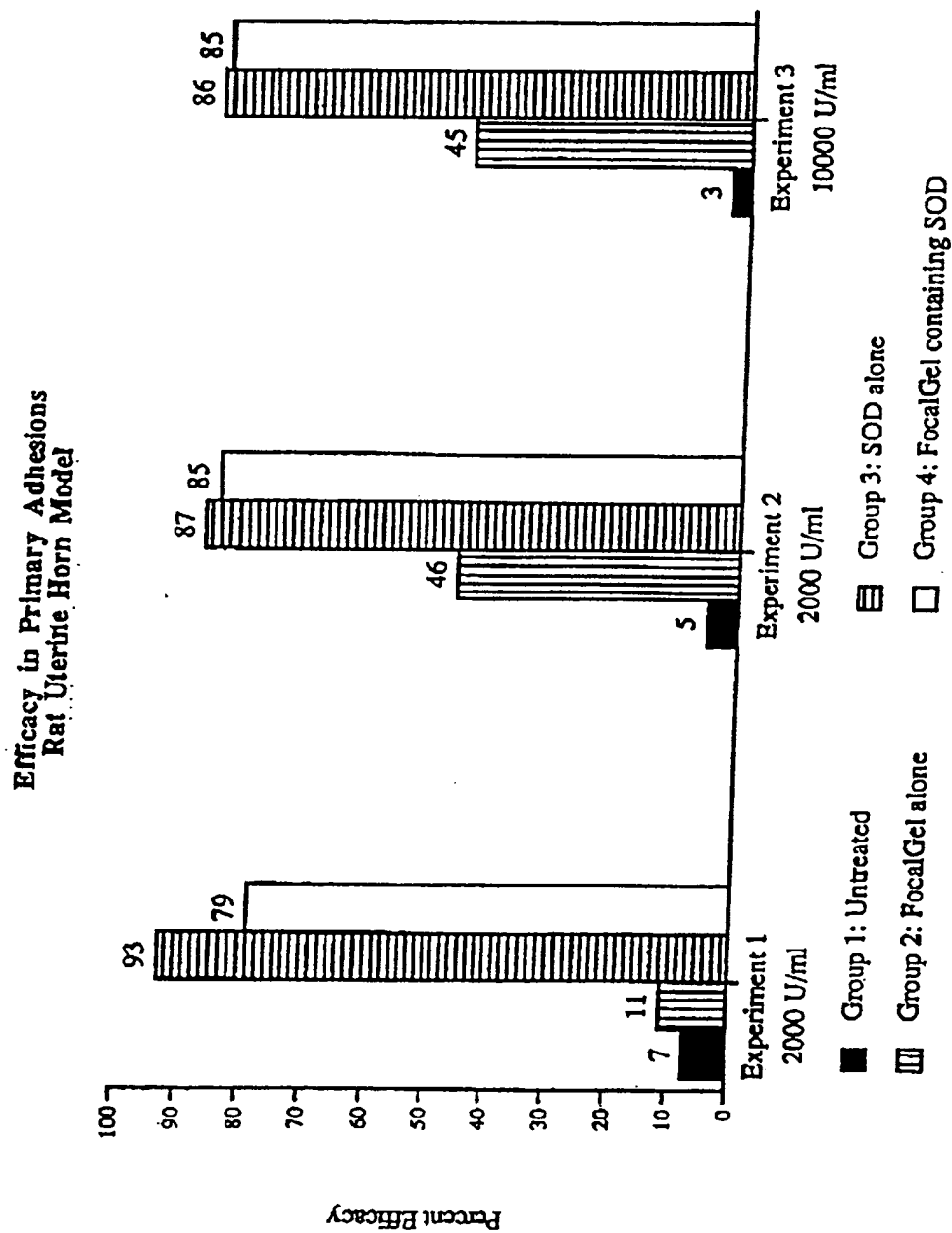
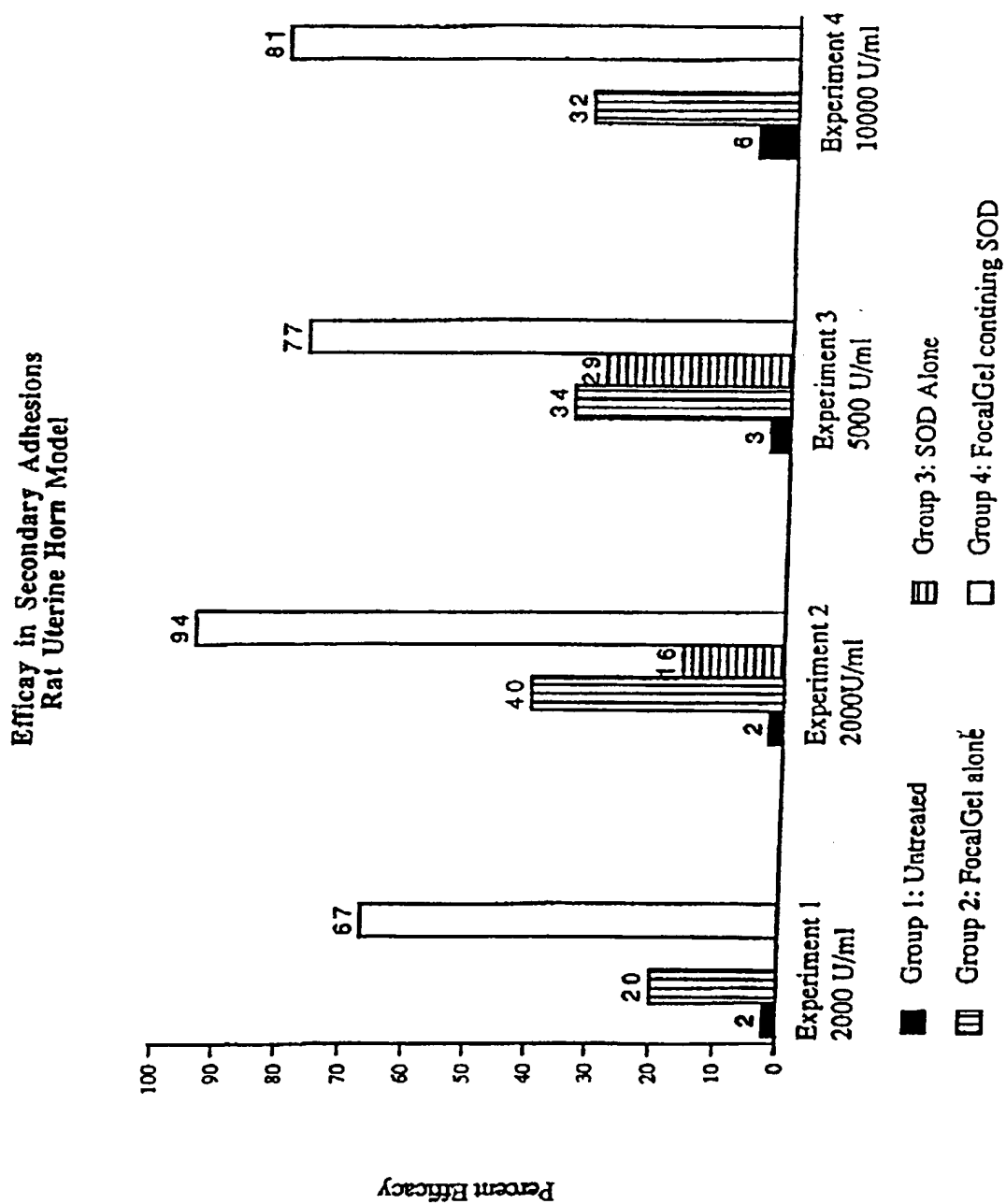# Best Available Copy

FIGURE 1

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FIGURE 2

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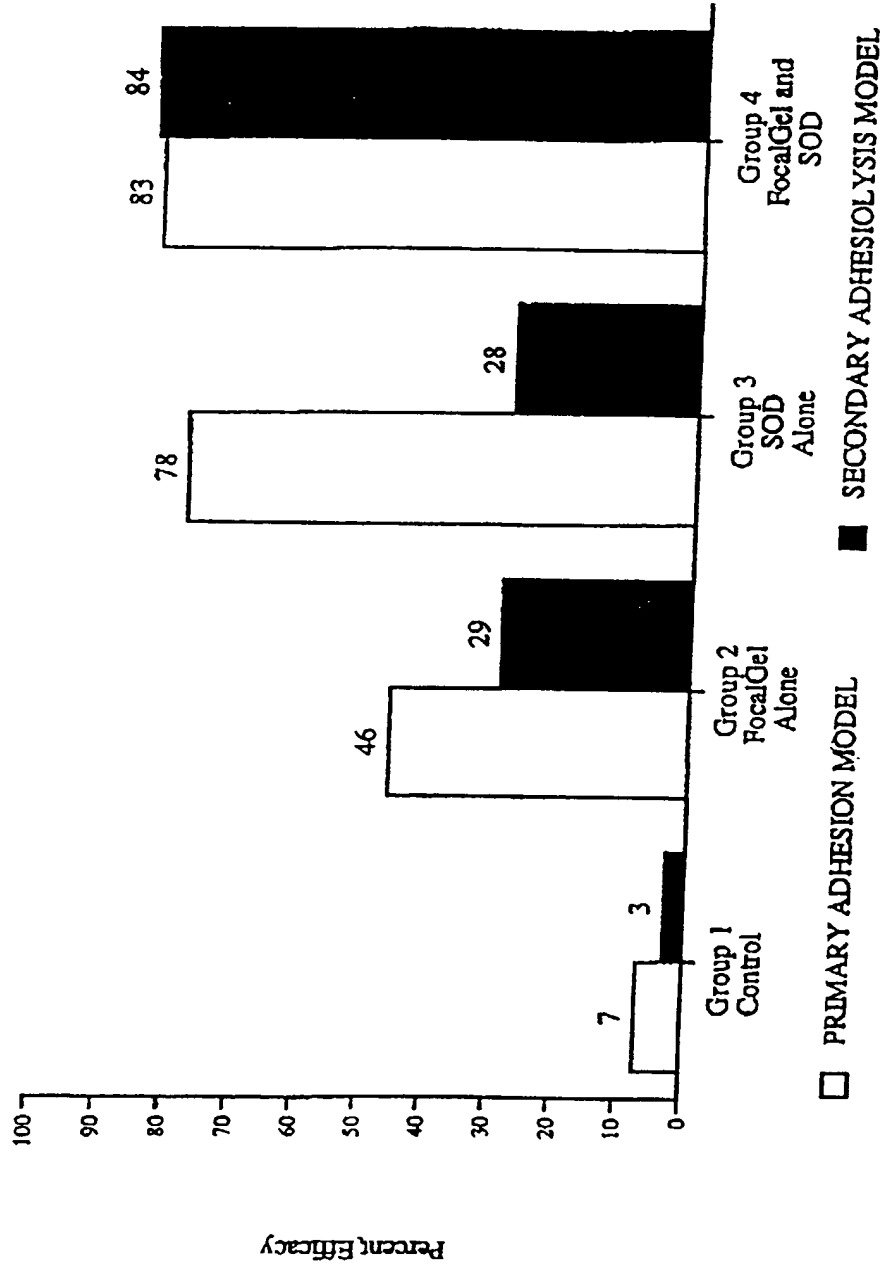
FIGURE 3

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   A61K9/00   A61K47/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 17669 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 16 September 1993 cited in the application see page 40 - page 41; example 12 see page 53 - page 54; example 19 ---	1-4, 7-14,17
Y	WO,A,93 16687 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 2 September 1993 see page 58 - page 59; example 25 ---	1-4, 7-14,17
A	US,A,4 806 621 (KOHN ET AL.) 21 February 1989 see column 4, line 3 - line 16 ---	1-17
A	US,A,4 371 519 (HETTINGER) 1 February 1983 see the whole document -----	1-17

☐	Further documents are listed in the continuation of box C.	☒	Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 1996	01.08.96

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Benz, K

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
    because they relate to subject matter not required to be searched by this Authority, namely:

    Remark: Although claim 17 is directed to a method of treatment of the

    human/animal body the search has been carried bout and based on the alleged
    effects of the composition.

2. [ ] Claims Nos.:
    because they relate to parts of the international application that do not comply with the prescribed requirements to such
    an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
    because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all
    searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment
    of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report
    covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is
    restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9317669	16-09-93	AU-B-	3780993	13-09-93
		CA-A-	2117584	02-09-93
		CA-A-	2117588	16-09-93
		EP-A-	0627911	14-12-94
		EP-A-	0627912	14-12-94
		JP-T-	7506961	03-08-95
		JP-T-	7507056	03-08-95
		NZ-A-	251039	26-03-96
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		WO-A-	9316687	02-09-93
		US-A-	5468505	21-11-95
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		NZ-A-	251039	26-03-96
		US-A-	5410016	25-04-95
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